CHAPTER 12

PROTEASE INHIBITORS: PHYSIOLOGICAL PROPERTIES AND NUTRITIONAL SIGNIFICANCE[1]

J. J. RACKIS

Northern Regional Research Center
Agricultural Research
Science and Education Administration
U.S. Department of Agriculture
Peoria, Illinois 61604

AND

M. R. GUMBMANN

Western Regional Research Center
Agricultural Research
Science and Education Administration
U.S. Department of Agriculture
Berkeley, California 94710

INTRODUCTION

The relative capacities of various sources of TI to inhibit growth and enlarge the pancreas vary widely. Multiple forms of protease inhibitors usually exist in foodstuffs. Immature, mature, and germinated soybeans, which are a primary source of protein for world needs, contain high levels of TI activity. Regardless of variety or stage of maturity, TI activity can be readily eliminated by ordinary cooking and moist-heat treatment.

Soybean protein products, properly processed, are an excellent source of low-cost, high-quality protein for human needs (Wilcke *et al.* 1979;

[1]Mention of firm names or trade products does not imply endorsement by the U.S. Department of Agriculture over firms or similar products not mentioned.

Reprinted from
Antinutrients and Natural Toxicants in Foods, R. L. Ory, ed.
Food & Nutrition Press, Inc., Westport, CT 06880 USA

American Oil Chemists' Society 1979). Food legumes, which include those species of the family Leguminosae (peas, beans, and soybeans) and other oilseeds, are the most important and economical sources of protein for human consumption (PAG 1973). In 1970, world production of major oilseeds and legumes that contain 20—45% protein was nearly 150 million metric tons (Food and Agriculture Organization 1970).

Ever since Osborne and Mendel (1917) observed that soybeans would not support the growth of rats unless the beans were cooked for 3 h on a steam bath, the nature of the substances that inhibit growth, enlarge the pancreas, and cause other physiological and biochemical effects in various species of animals has been investigated extensively (Rackis 1974; Anderson *et al.* 1979). The observation by Osborne and Mendel (1917) led to the discovery of a large number of heat-labile and heat-stable substances in many plant foodstuffs (Liener and Kakade 1969; Committee on Food Protection 1973). With the development of new technology and the manufacture of a wide variety of soy protein products, precise conditions of heat treatment required to inactivate the antinutritional factors in soybeans have been described (Smith and Circle 1972; American Soybean Association 1974). A series of papers on improving protein quality of selected legumes and on feeding trials with poultry, pigs, and ruminants has been reported (Commission of the European Communities 1977).

For maximum conversion of the raw proteins of soybeans and other food legumes into products with good nutritional quality, the conditions of heat treatment must inactivate the antinutritional substances as well as transform the raw protein into a more readily digested form (Kakade *et al.* 1973). Legumes are generally deficient in the sulfur-containing amino acids, methionine and cystine. Bioavailability of methionine and cystine in autoclaved navy beans is low (Evans and Bauer 1978). Following heat treatment, the major proteins of navy beans are less susceptible to *in vitro* enzymatic hydrolysis than is bovine serum albumin similarly treated (Romero and Ryan 1978). Several workers have reported the existence in legume proteins of either enzyme-resistant peptides or protein components that showed little susceptibility to proteolytic digestion (Fukushima 1968; Seidl *et al.* 1969; Boonvisut and Whitaker 1976; Bressani *et al.* 1977). Thompson and Liener (1978) reported that *in vivo* digestibility of raw navy bean protein, free of trypsin inhibitor, was very poor.

The extent to which protease inhibitors of soybeans are responsible for the antinutritional effects of raw and under-processed soy protein products and the mechanism by which protease inhibitors inhibit growth and enlarge the pancreas will be evaluated. The practical significance of residual inhibitor activity in heat-processed soy protein products and the

biochemical effects of other protease inhibitors will be discussed. An evaluation of the morphological changes that occur during enlargement of the pancreas will not be made here.

BIOLOGICAL-PHYSIOLOGICAL FACTORS IN RAW FOODSTUFFS

Sources of Protease Inhibitors

A number of deleterious effects occur in monogastric animals fed raw soybeans, dehulled, defatted soybean meal, and meal fractions having high trypsin inhibitor (TI) activity (Table 12.1). The heat-labile effects are physiologically interrelated. In general, hypersecretion of pancreatic enzymes is initiated by protease inhibitors and undenatured protein in raw soy flour and other food legumes. Acceleration of enzyme activity results from the formation of complexes with the digestive enzymes, trypsin and chymotrypsin, in the intestinal tract. Chronic stimulation leads to pancreatic hypertrophy and growth inhibition.

TABLE 12.1
PROPERTIES OF HEAT-LABILE FACTORS IN SOYBEANS

Inhibit growth	Enhance pancreatic synthesis of protein, phospholipid, nucleic acid
Inhibit proteolysis	Stimulate pancreatic enzyme secretion
Reduce protein digestibility	Stimulate bile secretion
Increase sulfur amino acid requirements	Reduce metabolizable energy
Enlarge pancreas	

Purified Kunitz soy TI's (Lyman *et al.* 1962; Rackis 1965) and the soy Bowman-Birk inhibitor, which inhibits chymotrypsin to a greater extent than trypsin, also enhance pancreatic enzyme secretion, enlarge the pancreas, and inhibit rat growth, but not to the same extent as raw soybean meal (Konijn *et al.* 1970; Gertler *et al.* 1967). Other trypsin and chymotrypsin inhibitors stimulate the pancreas and inhibit growth (Melmed and Bouchier 1969; Rackis 1972, 1974; Niess *et al.* 1972). These include: lima beans, kidney beans, field beans, potatoes, egg white, bovine pancreas, and the synthetic TI, p-amino-benzamide. Peanut TI's (Kwann *et al.* 1968) inhibit rat growth and cause pancreatic hypertrophy, whereas those in Hyacinth beans (Dolichos Lablab) (Phadke and Sohonie 1962) and opaque-2-corn (Mitchell *et al.* 1976) do not. TI activity of opaque-2-corn is twice the level of normal corn (Swartz *et al.* 1977). Partially purified TI's have been isolated from rice bran (Tashiro and Maki

1978) and alfalfa (Chang *et al.* 1978), but their nutritional effects in animals are not known. Opaque-2-corn, rice hulls, and alfalfa contain relatively high levels of TI with respect to protein content. Dehydrated soluble potato solids inhibit chick growth (Gerry 1977) and contain 58 trypsin inhibitor units per mg sample, compared to 72 and 100 units in commercial and laboratory-prepared raw defatted soy flour, respectively (Kakade *et al.* 1974). Cholecystokinin (CCK), a hormone in the duodenal mucosa that regulates protein digestion and pancreatic activity, also causes pancreatic hypertrophy, inhibits growth, and inhibits trypsin (Rackis 1974).

Pancreatic Enzyme Profile Patterns

In general, the pancreases of animals fed diets containing various types of protease inhibitors have much higher levels of protein, nucleic acids, and enzymes after a fast than comparable fasted animals fed control diets. When enzyme levels were measured after the animals were fed, those receiving protease inhibitors generally had lower levels of pancreatic enzymes than animals not receiving the inhibitor (Niess *et al.* 1972). Protease inhibitors stimulate protein synthesis and enzyme secretion from the pancreas. Inhibition of proteolysis, the presence of undigested protein in the intestinal tract, and a decreased release of amino acids in raw soy diets induce a compensatory reaction in the pancreas and a general stimulatory effect on other endogenous secretions. These effects (see also Table 12.1) would explain the interrelationship between depressed metabolizable energy, poor fat absorption, and changed carbohydrate metabolism in rats and chicks fed raw soybeans (Rackis 1974). The addition of 0.5% Kunitz soybean TI to toasted soybean meal diets results in elevated levels of nitrogen extending from the small intestine to the cecum. Pancreatic enzyme activity remains elevated throughout the intestinal tract (Lyman 1957). Such effects are similar to those observed with animals fed raw soybean meal (Lyman 1957; Bielorai *et al.* 1977; Nitsan and Madar 1978). These studies indicate that much of the elevated nitrogen and enzymatic activity is of pancreatic origin.

Rackis (1972, 1974) and Dijkhof *et al.* (1977) have summarized the complex changes that occur in synthesis and secretion of pancreatic enzymes following the ingestion of raw soybean meal, soybean whey fractions, and purified TI's. The considerable differences in enzymatic profile and relative rates of synthesis of specific enzymes and nonparallel secretions of specific pancreatic enzymes have been attributed to such factors as age, feeding conditions, level and type of ingested protease inhibitor, and the method used to measure enzyme activity (Dijkhof *et al.* 1977; Dijkhof and Poort 1978). In comparing the relative rates of pancreatic

protein synthesis brought about by feeding protein-rich, carbohydrate-rich diets or raw soybean meal, the overall adaptation to protein-rich and soybean diets was similar in that there is an increased synthesis of proteases and decreaded synthesis rate of amylase (Dijkhof *et al.* 1977). Enzymatic activities in the intestinal tract of chicks fed TI's from soybeans and egg white were similar, and both TI's caused pancreas enlargement (Nitsan and Gertler 1972).

Several studies indicate that the regulation of pancreatic protein synthesis and secretion in different animal species in response to protease inhibitors, CCK, and other dietary secretogues involves complex digestive compensation (Schneeman *et al.* 1977; Folsch *et al.* 1974; Johnson *et al.* 1977; Yen *et al.* 1977; Snook 1968; Folsch and Wormsley 1974, 1976; Corring and Bourdon 1977).

Species Differences

Effects of raw soybean meal and TI diets on growth and pancreases of various animals are summarized in Table 12.2. The only report on man (Lewis and Taylor 1947) indicates that nitrogen balance in two adults fed 180 g of raw soy flour was 80% of that with autoclaved flour. Pancreatic hypertrophy is a sensitive physiological response in young rats (Rackis 1965). Oral administration of just 2 mg Kunitz soybean TI per g body weight per day greatly enhanced synthesis of nucleic acids, phospholipids, and protein in the pancreas of neonatal rats (Melmed *et al.* 1976). The pancreas of the suckling rat may be more sensitive than that of young weanling rats to the trophic stimulation by the TI. Furthermore, extensive biochemical changes occurred in the pancreas before significant differences in weight gain were observed in rats fed TI and control diets (Melmed *et al.* 1976).

Pancreatic hypertrophy occurs in broiler chickens fed raw field beans (*Vicia faba*) (McNab 1977). Heat treatment will improve the nutritive value of field beans and other food legumes, with significant improvements occurring in rats and chicks, but not in pigs (Kakade *et al.* 1969; Liener 1976; Commission of the European Communities 1977).

In the chick intestinal proteolysis is inhibited to a greater extent than in the rat and the compensatory pancreatic secretion to counteract the effect of the TI is not as great. Enlargement of the pancreas occurs in the rat and chick. In the pig, dietary soybean meal and TI inhibit growth but do not cause pancreatic hypertrophy even though pancreatic enzyme activity is increased (Yen *et al.* 1977). Inhibition of intestinal proteolysis may be the primary cause of growth inhibition, since there is no pancreatic hypertrophy in the pig.

Feeding of raw soybean meal to calves inhibited growth in the absence

TABLE 12.2
BIOLOGICAL EFFECTS OF RAW SOYBEAN MEAL IN VARIOUS ANIMALS[1]

Species	Growth Inhibition	Pancreas Size	Pancreas Enzyme Secretion
Rat[2]	+[3]	+	+
Chicken[2]	+	+	+
Pig[2]	+	—	±
Calf	+	—	±
Dog	—	—	[4]
Human	[5]	[5]	[5]

[1]Rackis (1974).
[2]Although adult animals maintain body weight, pancreas effects still occur.
[3]+ = growth inhibition and pancreatic hypertrophy and hypersecretion; = no effect; ± = hyposecretion.
[4]Hyposecretion initially; normal after continued feeding.
[5]Unknown. However, two adults, in a 9-day feeding trial had positive nitrogen balance for both raw and autoclaved soy flour.

of pancreatic hypertrophy; purified soybean TI exerted no deleterious effects (Kakade *et al.* 1976). Normal pancreas weights and pancreatic exocrine function occur in dogs fed 15% raw soybean meal for 16 weeks (Patten *et al.* 1971A,B). Pancreatic hypertrophy occurs in the young and adult chicken, turkey, rat, and mouse.

Pancreatic Hypertrophy-Growth Inhibition: Mechanism

Growth inhibition in young animals is brought about by an excessive fecal loss of protein secreted by the pancreas. Since pancreatic enzymes are rich in sulfur-containing amino acids, the endogenous loss of protein cannot be compensated by dietary soy protein, which like most vegetable proteins is first limiting with respect to cystine plus methionine. Supplementation of raw soybean meal diets with essential amino acids will counteract growth inhibition but not pancreatic hypertrophy (Booth *et al.* 1960). In the adult animal, because of a lower amino acid requirement, there is no loss of weight; but pancreatic hypertrophy occurs.

The mechanism whereby the TI's inhibit growth and enlarge the pancreas is still not fully understood, but the essential characteristics are illustrated in Fig. 12.1.

Experiments with rats have demonstrated that pancreatic enzyme secretion is controlled by a negative feedback mechanism (Green and

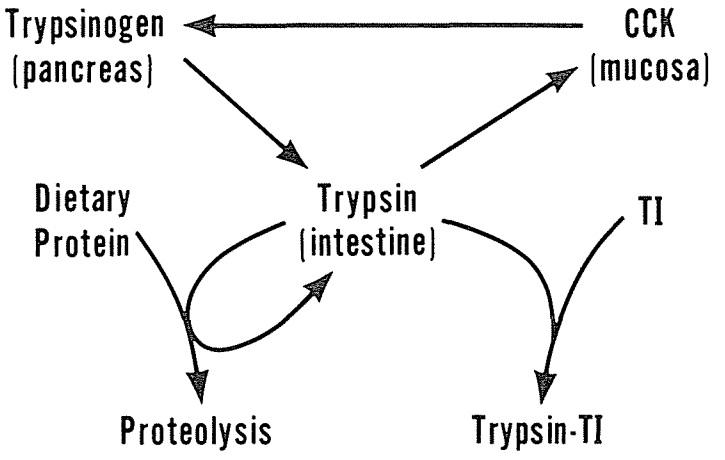

FIG. 12.1. REGULATION OF TRYPSIN SECRETION
CCK, cholecystokinin; TI, trypsin inhibitor.

Lyman 1972; Green *et al.* 1973). The amount of pancreatic secretion is determined by the level of free trypsin and/or chymotrypsin present in the intestine. As the level of trypsin goes below a threshold level, the pancreas is induced to produce more enzymes. The TI's evoke increased pancreatic enzyme secretion by forming inactive trypsin-TI complexes, thereby decreasing the suppression exerted by free trypsin. Dietary protein also forms a complex with trypsin during ingestion, thereby decreasing the threshold level of free trypsin even further and resulting in more pancreatic secretion into the intestinal tract (Schneeman *et al.* 1977). Poorly digested protein, such as in raw soybean meal and other legumes (Kakade *et al.* 1973; Evans and Bauer 1978; Bressani *et al.* 1977; Thompson and Liener 1978), would have an even greater effect in stimulating enzyme secretion.

The trophic response of the pancreas to dietary TI is an indirect response that is initiated in the intestine and not in the blood (Schneeman and Lyman 1975; Schneeman *et al.* 1977). Apparently, dietary TI has no humoral activity, since Madar *et al.* (1977) reported that only negligible amounts of soybean Bowman-Birk TI (molecular weight approximately 8000) could be detected in the blood. Most likely CCK is directly responsible for the stimulation of the pancreas and selective changes in the pancreatic enzyme synthesis and secretion. Decreasing the intestinal level of trypsin and chymotrypsin, mediated by the formation of complexes with protein and TI's, results in the release of CCK from binding sites in the intestinal mucosa (Green and Lyman 1972; Green *et al.* 1973). The release

of CCK is inhibited by free trypsin (Wilson *et al.* 1978). Repeated injections of CCK, which also has TI activity, cause pancreatic hypertrophy and inhibit growth.

Pancreatic feedback inhibition occurs in humans (Ihse *et al.* 1977) and pigs (Corring 1974) but not in dogs (Schneeman *et al.* 1977; Sale *et al.* 1977). However, the physiological response of the young pig to raw soybean meal is reported to be different in several respects from that of the growing rat and chick (Yen *et al.* 1977; Corring and Bourdon 1977).

Nutritional Significance in Humans

TI's and dietary protein apparently stimulate pancreatic activity by a common mechanism—it is only the extent of stimulation that differs. The feedback mechanistic concept reinforces evidence that residual TI in toasted edible-grade soy protein products may have no nutritional significance as long as the TI level remains below the biological threshold level at which pancreatic hypertrophy occurs (Rackis *et al.* 1975A; Rackis *et al.* 1979).

The practical significance of TI's with respect to human nutrition at the moment is speculative. Since pancreatic feedback inhibition occurs in humans and since only 30% of human trypsin is inhibited by an equivalent weight of soy TI, higher TI levels may be needed to counteract the suppression of human pancreatic enzyme secretion by trypsin in the intestinal tract. Bovine trypsin, which is generally used for assays of TI activity, is inhibited by an equivalent amount of Kunitz soy TI. The predominant cationic form of human trypsin is only weakly inhibited by soybean TI, whereas the anionic form is inhibited completely (Figarella *et al.* 1974). Rat trypsins are inhibited by soy TI, but the extent of inhibition has yet to be quantitated. That there is a causal relationship between the extent of *in vitro* inhibition of trypsin and pancreatic hypertrophy *in vivo* has been largely assumed.

In further support of the probability that soybean TI may have less significance in humans than in rats, Liener (1977) discusses the interesting relationship between the pancreas size of various species of animals and their sensitivity to pancreatic hypertrophy induced by raw soybean meal and TI. Animals whose pancreas weight is greater than 0.3% relative to body weight exhibit hypertrophy when fed raw soybeans, whereas those whose relative weight is less than 0.3% do not. In man, size of the pancreas is 0.09 to 0.12% relative to body weight. If size of the pancreas reflects its functional activity, as suggested by Goss (1966), then the physiological response to raw soybeans or TI's would likely differ in various animal species.

RELATIONSHIP BETWEEN GROWTH INHIBITION AND PANCREATIC HYPERTROPHY OF SOY PROTEIN PRODUCTS AND PURIFIED TI

Whole Soybeans

By live steam treatment, a process referred to as toasting, nutritive value of soy flour can be raised to values nearly equal to that of animal protein (Fig. 12.2). The relationship between destruction of TI activity and the increase in protein efficiency ratio (PER) of defatted soy flakes as a function of toasting is illustrated in Fig. 12.3. Associated with the increase in nutritive value is the inactivation of TI, elimination of pancreatic hypertrophy, and simultaneous conversion of raw refractory proteins to more readily digestible forms (Rackis *et al.* 1975A; Kakade *et al.* 1973).

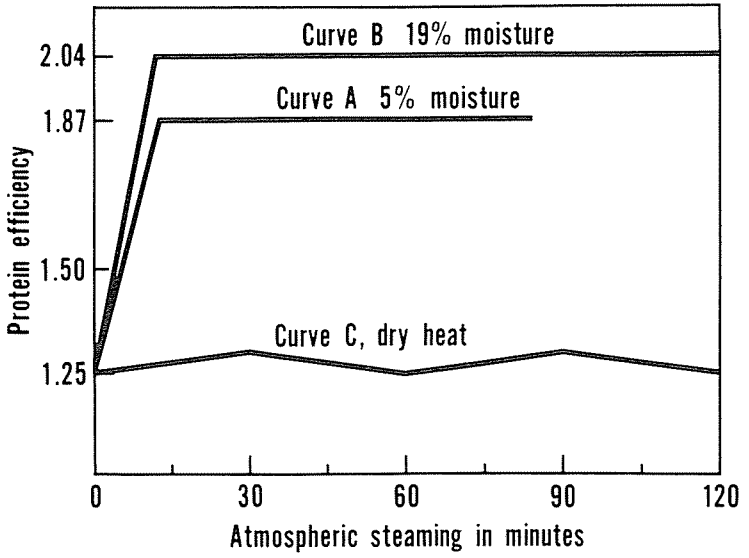

FIG. 12.2. EFFECT OF MOISTURE AND TYPE OF HEATING ON PROTEIN EFFICIENCY VALUES OF DEFATTED SOYBEAN MEAL. CONDITIONS: LIVE STEAM AT ATMOSPHERIC PRESSURE, 100°C; CURVE A, PROTEIN EFFICIENCY VALUES OF MEAL WITH 5% MOISTURE PRIOR TO AUTOCLAVING; CURVE B, PROTEIN EFFICIENCY VALUES OF MEALS WITH 19% MOISTURE INITIALLY; CURVE C, DRY HEAT AT 120°C (SMITH *ET AL.* 1964)

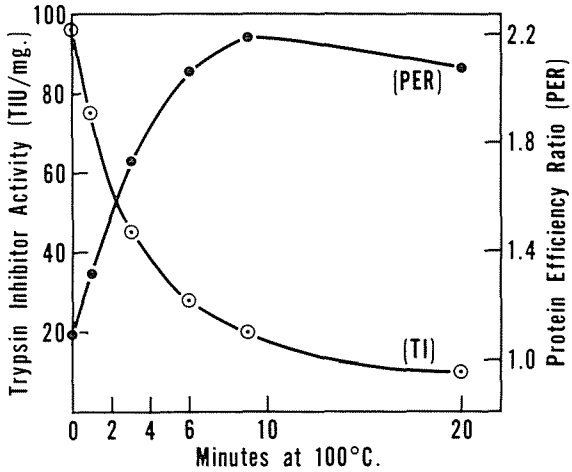

FIG. 12.3. EFFECT OF STEAMING ON TRYPSIN INHIBITOR ACTIVITY AND PROTEIN EFFICIENCY RATIO OF DEFATTED SOY FLOUR

TIU = trypsin inhibitor units as defined by Kakade *et al.* (1969). Data: Rackis *et al.* 1975A.

Particle size, initial moisture, time, temperature, and pressure are the major factors influencing cooking rates, inactivation of TI, and subsequent increase in nutritive value. Extrusion-cooking, infrared heating, microwave processing, and other forms of food processing are all equally effective in improving protein quality, provided precise control of the cooking process is maintained (Rackis 1974; Rackis *et al.* 1975B).

Kakade *et al.* (1972) analyzed over 100 varieties and strains of soybean. TI activity ranged from 66—233 TI units/mg protein. Raw flours prepared from these varieties inhibited growth and caused pancreatic hypertrophy. As indicated in Table 12.3, PER's were markedly improved after autoclaving, and pancreas weights of rats fed heated soybeans were not significantly different from casein-fed rats.

Rackis (1978) compiled data from several sources on TI content in soybean varieties and in immature and germinated soybeans. As shown in Table 12.4, no heat stable TI's are present in soybeans of varying maturity, and PER's are greatly improved by autoclaving. Although Bates *et al.* (1977) reported that TI activity decreased about 70% in a 4-day germination period, others reported that TI activity of germinated soybeans decreased very little.

TABLE 12.3
NUTRITIVE VALUE AND TRYPSIN INHIBITOR CONTENT OF DIFFERENT VARIETIES AND STRAINS OF SOYBEANS[1]

Sample	Protein (%)	Raw Flour TIU[2]/mg Protein	PER[3] Raw	PER[3] Toasted[4]	Pancreas Weight Raw	Pancreas Weight Toasted
Disoy	39.6	100	1.47	2.66	+[5]	—[5]
Provar	41.2	106	1.60	2.20	+	—
PI 153319	36.6	168	0.88	2.46	+	—
Hark	39.1	100	1.21	2.32	+	—
PI 153206	37.5	139	1.21	1.95	+	—

[1]Kakade *et al.* (1972).
[2]Trypsin inhibitor units.
[3]Protein efficiency ratio (gram weight gain per gram protein consumed). Data adjusted to a basis of PER = 2.50 for casein.
[4]Autoclaved at 15 psi (120 °C for 30 min.
[5]+ = Pancreatic hypertrophy; — = normal compared to casein; pancreas weights were negatively correlated with PER in rats fed raw soy.

TABLE 12.4
EFFECT OF MATURATION AND GERMINATION ON TRYPSIN INHIBITOR ACTIVITY AND NUTRITIVE VALUE OF SOYBEANS[1]

Stage of Maturity	Trypsin Inhibitor Activity[2,3] Raw	Trypsin Inhibitor Activity[2,3] Heated[4]	PER[5] Raw	PER[5] Heated
Immature	49.0	1.5	0.77	2.05
Mature	52.2	0.6	0.75	2.11
Germinated	17.8	1.7	0.64	2.02

[1]Bates *et al.* (1977).
[2]Change in absorbance at 256 nm/min/g protein compared to control with no trypsin inhibitors, Bragg soybeans.
[3]Activity in extracts under conditions employed.
[4]Autoclaving 121 °C for 15 min.
[5]Protein efficiency ratio corrected on a basis of PER = 2.50 for casein.

Soy Meal Fractions

Soybean meal can be processed into several protein products (Smith and Circle 1972). Nutritive value and presence of growth-inhibiting and pancreatic hypertrophic factors in various fractions of soybeans, obtained during the processing of meal into protein concentrates and isolates, have been studied in detail (Rackis *et al.* 1963; Garlich and Nesheim 1966; Sambeth *et al.* 1967). The greatest inhibition of growth

and enlargement of the pancreas in rats and chicks occurred with meal fractions having high trypsin inhibitor activity. These same fractions as well as purified TI's contract the gallbladder and accelerate bile secretion in chicks (Sambeth *et al.* 1967; Niess *et al.* 1972) and produce many of the effects listed in Table 12.1. Soybean whey, a byproduct in the manufacture of protein isolates, contains most of the heat-labile antinutritional factors (Rackis *et al.* 1963; Garlich and Nesheim 1967). The protein quality of the whey proteins after toasting is greater than that of meat and milk because of an excellent balance of essential amino acids (Rackis *et al.* 1971).

When raw whey proteins are added to a casein diet, at a level equal to the TI activity of a diet containing raw soybean meal as the sole source of protein, they inhibited rat growth, reduced protein efficiency and enlarged the pancreas to the same extent as with raw meal (Rackis 1965). The whey proteins account for at least 80% of the TI activity of raw soybean meal.

Crystalline TI Experiments

A series of experiments was conducted to determine quantitatively the relative ability of crystalline Kunitz soybean TI to inhibit growth and cause pancreatic hypertrophy in rats compared with raw meal containing equivalent TI activity (Table 12.5). The Kunitz TI accounts for about 50% of the total TI activity in raw meal. Maximum pancreatic hypertrophy occurs with raw meal diets containing 10% raw meal, which supplies 0.4% TI (Diet 2) and 5% of the protein in the diet. Except for diet 4, casein was added to all soy diets to maintain dietary protein levels at 10%. Compared with the casein control diet, about 17% raw meal (diet 3) was required to produce the greatest reduction in weight gain and protein efficiency. The TI content of the diet was 0.68%. Higher levels of raw meal (diet 4) begin to reverse growth inhibition but not pancreatic effect.

In diet 5, crystalline TI at about the same level as in diet 2 reduced weight gain and lowered protein efficiency, but not to the same extent as raw meal (diet 2). However, maximum pancreatic hypertrophy was obtained. In comparing diet 6 containing purified TI with diet 3 containing the same level of TI activity as raw meal, the decrease in weight gain of rats on diet 6 was 66% of that obtained with diet 3; the TI accounted for 56% of the reduction in protein efficiency associated with raw meal diets.

These results demonstrate that not all of the growth-inhibiting effects of raw soybeans can be attributed to the TI's. Kakade *et al.* (1973) fed rats crude soybean extracts from which the TI activity was selectively removed by affinity chromatography. By comparison with groups of rats fed the original extract as well as the heat-treated extract, they concluded

TABLE 12.5
RELATIONSHIP BETWEEN RAW SOYBEAN MEAL AND TRYPSIN INHIBITOR ON RAT GROWTH, PROTEIN EFFICIENCY, AND PANCREAS[1]

Diet no.	Diet[2]	% Inhibitor in Diet	Mean Weight Gain ± SE[3], g	% of Control	PE	Mean Pancreas Weight ± SE, g/100 g BW
1	Control, 10%	—	65.4 ± 7.04	—	2.48	0.60 ± 0.22
2	Raw meal, 10%	0.40	35.6 ± 3.97[4]	54.5	1.54	0.79 ± 0.04[4]
3	Raw meal, 17%	0.68	24.6 ± 5.44[4]	31.6	1.13	0.77 ± 0.03[4]
4	Raw meal, 23%	0.97	35.8 ± 3.22[4]	54.7	1.24	0.85 ± 0.03[4]
5	STI[5]	0.45	40.2 ± 5.50[6]	61.5	1.76	0.74 ± 0.02[4]
6	STI	0.63	38.4 ± 4.00[4]	58.7	1.72	0.74 ± 0.04[4]

[1]Rackis (1965).
[2]All diets contain 10% protein, except diet 4 which is 12.3% protein; 35-day assay.
[3]SE = Standard error; PE = protein efficiency; BW = *body weight.*
[4]$P < 0.01$.
[5]Crystalline Kunitz soybean trypsin inhibitor.
[6]$P < 0.05$.

that about 40% of the growth-inhibiting and pancreatic hypertrophic effects of the original extract was attributed to the TI's. Growth inhibition and enlargement of the pancreas with raw extracts in the absence of the TI's was attributed to the poor digestibility of the raw undenatured protein.

Another series of feeding experiments was conducted to determine the influence of protein level in the diet on the ability of TI to inhibit growth and enlarge the pancreas (Table 12.6). There is a significant reduction in weight gain and protein efficiency ratio in rats fed 0.6% TI in a 10% casein diet (diet 8), whereas in diet 11 containing 14% casein, the purified TI had little effect on growth or protein efficiency. Regardless of the dietary protein level, pancreatic hypertrophy occurred in all rats fed crystalline Kunitz soybean TI. The greatest increase in pancreatic hypertrophy occurred with diet 11, which contained higher protein levels. If the size of the pancreas reflects its functional activity (Goss 1966), the increase in pancreatic hypertrophy observed with rats fed soybean TI in diets containing 14% casein, compared with a dietary protein level of 10%, would be associated with an enhanced secretion of pancreatic enzymes (Schneeman *et al.* 1977). Diets containing amino acids or hydrolyzed casein evoke a small pancreatic response that is similar to the response of a protein-free diet. Intragastric infusion of a diet containing 18% casein stimulated pancreatic secretion 3- to 4-fold higher than amino acid or hydrolyzed casein diets. Addition of 0.75% purified soybean TI to the casein diet increased total pancreatic protein output nearly twice that of rats fed the 18% casein diet without soy TI (Schneeman *et al.* 1977).

TABLE 12.6
EFFECT OF PROTEIN LEVEL ON GROWTH, PROTEIN EFFICIENCY, AND PANCREAS OF RATS FED SOY TRYPSIN INHIBITOR (TI)[1]

Diet no.	Diet[2]	Inhibition in Diet	Mean Weight Gain, g	PE[3]	Mean Pancreas Weight g/100 g BW[3]
7	Casein, 10%	0	95.2	3.04	0.51
8	Casein + TI[4]	0.6	79.0[6]	2.69[6]	0.62[6]
9	Raw soymeal	0.6[5]	54.6[6]	1.86[6]	0.69[6]
10	Casein, 14%	0	105.0	2.43	0.53
11	Casein + TI[4]	0.6	92.4	218	0.78[6]

[1]Rackis (1965).
[2]Diets 7 to 9, 10% protein; diets 10 and 11, 14% protein.
[3]PE = Protein efficiency; BW = body weight.
[4]Crystalline Kunitz trypsin inhibitor.
[5]TI from raw soybean meal.
[6]Statistically significant differences.

BIOLOGICAL THRESHOLD LEVELS OF TI'S

Soybeans—Short-Term Feeding Trials

Although recent evidence clearly indicates that TI's are one of the major factors responsible for the deleterious effects of raw soybean meal, there is a great amount of uncertainty regarding the biological threshold level of TI at which these biological effects occur. The question of whether the ingestion of low levels of residual activity in heat-processed soy products over a prolonged period would create adverse effects also remains unanswered.

To obtain such quantitative data, dehulled defatted soy flakes containing graded levels of TI were prepared by treating raw undenatured flakes with live steam at 100 °C for carefully controlled periods of time in a preheated autoclave. TI activity was determined by the procedure of Kakade *et al.* (1974). The TI assay is an official procedure of the American Oil Chemists' Society (1976) and American Association of Cereal Chemists (1974), designated methods Ba 11-74 and 71-10, respectively. Data on the inactivation of TI activity and reduction in protein solublity, in terms of NSI value, are given in Table 12.7 (Rackis *et al.* 1975A).

Four biological parameters were used in rat bioassays to evaluate the nutritive value of soy flours containing graded levels of TI: weight gain, PER, nitrogen digestibility, and pancreas weights. Results are given in Table 12.8. As the TI content of the diet decreased, body weight, PER, and nitrogen digestibility values increased to a maximum (diet 17) in rats fed a soy diet containing 212 mg TI/100 g diet and in which only 79% of

TABLE 12.7
NITROGEN SOLUBILITY INDEX (NSI) AND TRYPSIN INHIBITOR (TI) ACTIVITY OF HEAT-TREATED SOY FLOURS[1]

Heat Treatment min[2]	NSI	TI Activity TIU/mg[3]	TI Activity mg TI/g[4]	Destruction of TI Activity, %
0	97.2	96.6	50.8	0
1	78.2	74.9	39.4	23
3	69.6	45.0	23.7	54
6	56.5	28.0	14.7	71
9	51.3	20.5	10.8	79
20	37.9	10.1	5.3	90
30	28.2	8.0	4.2	92

[1]Source: Rackis *et al.* (1975A).
[2]Live steam at 100°C.
[3]As defined by Kakade *et al.* (1969).
[4]Calculated on the basis that 1 μg of trypsin inhibits an equivalent amount of TI, and 1 μg of "pure" trypsin has an activity of 1.9 TIU (Kakade *et al.* 1969).

the TI activity was destroyed. Additional heat treatment (diet 18) tended to lower nutritive value even though the TI content was further reduced.

Normal pancreas weights were obtained in rats fed samples in which only 54% of original TI activity of raw soy flour was destroyed (diet 15). Apparently, the rat can tolerate relatively high levels of TI (464 mg/100 g diet) before pancreatic hypertrophy occurs in short-term feeding trials.

These data further support the conclusion that the TI's make a significant contribution to the antinutritional effects of raw meal. In agreement with Kakade *et al.* (1973), it would appear that once the pancreatic hypertrophic effect of the TI has been eliminated (diet 15), the further increase in nutritive value of soy flour with continued cooking can be accounted for by an increase in nitrogen digestibility.

Soybeans—Long-Term Feeding Tests

Edible-grade, commercially manufactured soy protein products containing varying levels of residual TI activity were fed to rats from weaning to adulthood, a period of up to 300 days. Biological parameters evaluated were: body weight gain, organ weights, size and histology of the pancreas. Composition of the corn-casein control and corn-soy experimental diets is given in Table 12.9.

As shown in Fig. 12.4, the ability of soy flour, concentrate, and isolate to provide optimum growth in young rats and to maintain body weight in adults varied widely. Rats fed soy diets initially grew at a rate equal to or greater than those fed a comparable corn-casein control diet; but, with

TABLE 12.8
BIOCHEMICAL EFFECTS OF DEFATTED SOY FLOUR CONTAINING GRADED LEVELS OF TRYPSIN INHIBITOR (TI) IN RATS[1]

Diet no.	Dietary Protein[2]	TI Content mg/100 g Diet	Mean Body Weight (g) ± Standard Deviation	PER[3]	Nitrogen Digestibility[4]	Pancreas Weight ± Standard Deviation g/100 GBW[5]
12	Casein (0)	0	157 ± 16ab[6]	2.50	92	0.48 ± 0.03c[6]
13	Soy (0)	1001	84 ± 4f	1.13	74	0.68 ± 0.11a
14	Soy (1)	774	94 ± 8ef	1.35	78	0.58 ± 0.01b
15	Soy (3)	464	123 ± 5d	1.75	77	0.51 ± 0.06c
16	Soy (6)	288	141 ± 12c	2.07	83	0.52 ± 0.04c
17	Soy (9)	212	146 ± 11bc	2.19	84	0.48 ± 0.06c
18	Soy (20)	104	139 ± 13c	2.08	83	0.49 ± 0.05c
LSD[7]			11	0.17		0.06

[1]Rackis *et al.* (1975A).
[2]Time (min) of heat treatment at 100 °C is given in parentheses; 10% protein diet, 28-day feeding trial.
[3]PER = Protein efficiency ratio corrected on a basis of a PER = 2.50 for casein.
[4]Digestibility = intake-fecal nitrogen/intake × 100.
[5]GBW = grams body weight.
[6]Letters not in common denote statistical significance ($P < 0.05$).
[7]LSD = Least significant difference at the 95% confidence level.

TABLE 12.9
COMPOSITION OF CORN-CASEIN CONTROL AND CORN-SOY EXPERIMENTAL DIETS[1]

Ingredient, %	Casein	Diets[2,3] Flour	Concentrate	Isolate
Corn premix[4]	70.0	70.0	70.0	70.0
Casein	16.0	———	———	———
Soy flour (toasted)	———	28.0	———	———
Soy protein concentrate	———	———	22.3	———
Soy protein isolate	———	———	———	16.3
DL-methionine[5]	———	0.18	0.18	0.3
Dextrose	14.0	1.82	7.52	13.4
Protein content	19.7	19.9	19.6	19.9
Trypsin inhibitor content[6] mg/100 g diet	50.0	176.0	310.0	178.0

[1]Rackis *et al.* (1979).
[2]Soy ingredients were substituted for casein to maintain equivalent protein level, N × 6.25.
[3]Comparable diets were also formulated with added zinc: Soy flour, 25 ppm; soy concentrate and isolate, 50 ppm.
[4]Premix: yellow corn meal, 57.5; soy oil, 5.0; brewers yeast, 2.0; dehydrated alfalfa, 2.0; bone ash, 1.5; iodized salt, 0.5; vitamin A (2000 IU/g), and D_3 (200 units/g) mixture, 1.51 = 70%.
[5]Methionine supplementation calculated to achieve equivalent levels in all diets.
[6]Corn meal, soy flour, protein concentrate, and protein isolate contained 0.74, 5.6, 13.9, and 10.9 mg TI/100 g diet, respectively.

continued feeding for 237 days, body weights of rats fed the casein control were significantly greater than that of the rats fed soy flour. Those fed soy flour and protein isolate leveled off in growth at around 146 days, and body weight remained constant thereafter; whereas, the casein group continued to gain weight. The group fed soy protein concentrate began to lose weight by about the 174th day. However, when the diets were supplemented with vitamin B-12 at 237 days, there was little effect on growth with the casein control diet. Vitamin B-12 supplementation brought about an immediate stimulation of growth for all soy groups accompanied by a marked increase in feed consumption.

No pancreatic hypertrophy occurs in rats fed edible-grade soy flour, protein concentrates, and protein isolates from weaning to adulthood. TI content of the soy diets ranged from 178—310 mg/100 g. Gross and microscopic examination of pancreata revealed no abnormalities. No significant differences were found in organ weights between groups fed soy products and casein, except for increased kidney, liver, and testes weights relative to body weight only with the group fed soy protein concentrate in unsupplemented diets.

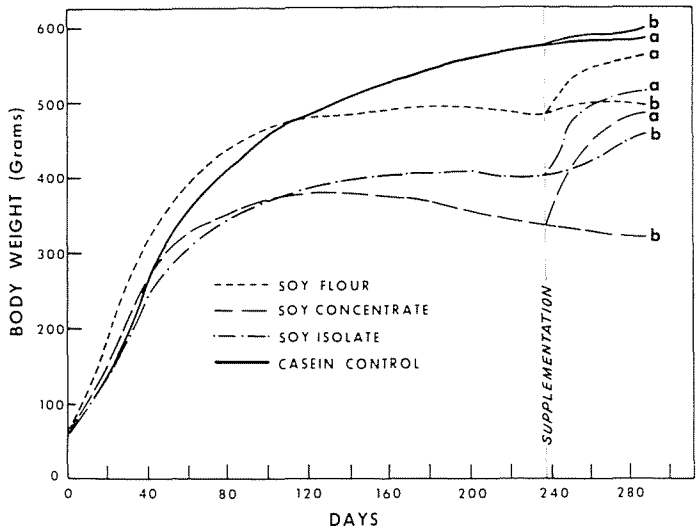

FIG. 12.4. GROWTH CURVES FOR RATS FED SOY AND CASEIN DIETS FROM WEANLING TO ADULTHOOD (UP TO 300 DAYS)

Curve B, unsupplemented diets, Series B; curve A, supplementation with complete vitamin-mineral mix or vitamin B-12 after 237 days feeding. Rackis *et al.* 1979.

Comparison with Other TI's

The relative capacity of various sources of TI's to inhibit growth and enlarge the pancreas is presented in Table 12.10. The soybean TI on a weight basis exhibited the greatest deleterious effect on growth and the pancreas. Bovine pancreatic TI was very active at a low concentration. The growth rate of each rat group was inversely related to the pancreatic response to the TI.

Diets containing 435 mg of TI/100 g diet, either as raw soy flour or purified Kunitz soybean TI, did cause pancreatic hypertrophy in growing pigs (Yen *et al.* 1977). Raw peanut flour diets, calculated to contain 250 mg TI/100 g of diet, caused pancreatic hypertrophy (Kwaan *et al.* 1968), whereas 106 mg of TI derived from corn meal did not (Mitchell *et al.* 1976). A diet containing 0.6% Kunitz soybean TI depressed growth of chicks and enlarged the pancreas (Garlich and Nesheim 1966).

REVERSIBILITY OF PANCREATIC HYPERTROPHY

Pancreatic hypertrophy in rats ingesting raw soybean meal for 38 days was reversed when the rats were switched to a casein control diet

TABLE 12.10
INFLUENCE OF PURIFIED TRYPSIN INHIBITORS (TI) ON GROWTH AND SIZE OF PANCREAS IN RATS (9-DAY TEST)

Rat Group	TI Content[1] %	Weight Gain, %	Pancreas Weight, g/100 g Body Weight
Control[2]	0	60	0.567
Soybean TI	0.6	34	0.811
Ovomucoid TI	0.6	44	0.642
Bovine pancreatic TI	0.043	52	0.670

Melmed and Bouchier (1969).
[1]Percent of diet.
[2]Protein content of diet 27%, of which 85% came from toasted full-fat soy flour.

containing 14% protein (Booth *et al.* 1964). In later feeding trials, Rackis *et al.* (1979) confirmed that TI-induced pancreatic enlargement is reversible.

In the later trial, two different basal diets were employed: the first was a commercial diet (Wayne) that contained several cereal and animal protein byproducts as well as some toasted soy flour, vitamins, and minerals; the second was a formulated corn-casein diet (Table 12.8). In the experimental diets, raw or toasted soy flour replaced 30% of the Wayne basal and was present at the 30% level in the casein-control diet, at the expense of casein and a portion of the dextrose.

Results given in Table 12.11 show that pancreas weights of the rats fed toasted soy diets for 35 days (diets 20 and 21) were not significantly different from the Wayne basal (diet 19). Diets containing 30% raw soy flour (diets 22 and 23) caused pancreatic hypertrophy and inhibited growth.

After 33 days on test, one-half of the rats fed raw soy were switched to the basal diet for an additional 71 days (diet 25). After continuous ingestion of soy flour for 104 days, rats fed raw soy had significantly greater pancreas weights (diet 24) than the group (diet 25) that was changed at 33 days from raw soy to Wayne basal. Note that body weight gains of the raw and toasted soy groups (diets 24 and 26) were not significantly different after 104 days. These results suggest that pancreatic hypertrophy is readily and completely reversible after 33 days of feeding raw soy.

The purpose of the second trial was to determine the relative capacity of defatted soy flours containing graded levels of TI activity to inhibit growth and cause pancreatic hypertrophy when fed to rats from weaning to adulthood. As in the first trial, soy flours were fed at the 30% level in

TABLE 12.11
GROWTH AND PANCREAS WEIGHTS OF RATS FED DEFATTED SOY FLOUR UP TO 104 DAYS AND REVERSIBILITY OF PANCREATIC HYPERTROPHY[1]

Dietary Group[2]	Body Weight Gain ± std. dev.[3] 0-33 Days[4]	33-104 Days	0-104 Days	Pancreas Wt. g/100 g Body Weight ± std. dev.
19 Wayne basal[5]	229 ± 25^{ABa}	———	———	0.47 ± 0.04^{Cc}
20 Toasted soy in Wayne basal	235 ± 20^{Aa}	———	———	0.50 ± 0.06^{Cc}
21 Toasted soy in corn-casein basal	242 ± 13^{Aa}	———	———	0.45 ± 0.04^{Cc}
22 Raw soy in Wayne basal	206 ± 21^{BCb}	———	———	0.77 ± 0.04^{Aa}
23 Raw soy in corn-casein basal	197 ± 17^{Cb}	———	———	0.63 ± 0.06^{Bb}
24 Raw soy in Wayne basal	189 ± 24^{Ab}	146 ± 30^{Bb}	335 ± 39^{Bb}	0.60 ± 0.06^{Aa}
25 Raw soy → Wayne basal[6]	201 ± 27^{Aab}	193 ± 12^{Aa}	394 ± 24^{Aa}	0.45 ± 0.05^{Bb}
26 Toasted soy in Wayne basal	221 ± 24^{Aa}	155 ± 29^{Bb}	376 ± 50^{ABa}	0.42 ± 0.05^{Bb}

[1]Rackis *et al.* (1979).
[2]Level of soy in diet, 30%, TI content (mg/100 g diet) toasted soy, 189; raw soy, 1410.
[3]Duncan's Multiple Range Test; means without a superscript letter in common are significantly different; P<0.05 = lower case; P<0.01 = upper case.
[4]0-35 days for groups 19-23.
[5]Commercial rat chow.
[6]Diet switched to Wayne basal after 33 days.

the corn-casein control diet (Table 12.9) at the expense of casein and a portion of the dextrose. Results are summarized in Table 12.12. Body weights of rats fed raw soy flour for 35, 168, and 215 days (diet 27) were significantly lower than the groups fed partly toasted (diet 29) and toasted soy flour (diet 31). The pancreas was also greatly enlarged in the raw soy group, although no histological abnormalities were noted in groups fed either raw, partly toasted, or toasted soy.

Rats fed raw soy for 35 days and then switched to a toasted soy diet showed improved growth and reversed pancreatic hypertrophy (comparing diets 27 and 28). These results show that high levels of toasted soy flour are as effective as a Wayne basal diet (see Table 12.11) in reversing pancreatic hypertrophy. Of special significance is the finding that continuous ingestion of high levels of TI (459 mg/100 g diet) did not inhibit growth nor cause pancreaic hypertrophy (diet 29) when compared to the

TABLE 12.12
LONG-TERM FEEDING OF DEFATTED SOY FLOUR CONTAINING GRADED LEVELS TRYPSIN INHIBITOR (TI) ON BODY AND PANCREAS WEIGHTS OF RATS[1]

Dietary Group no.	Days on Soy Diet[2] Raw	Partly Toasted	Toasted	TI Content mg/100 g Diet	Body Weights (± S.E.)[3] after 35 Days	168 Days[3]	215 Days	Pancreas Weight (± S.E.)[3] g/100 GBW
27	215	0	0	1269	251.4 ± 4.4^{Bb}	366 ± 10^{Cc}	432 ± 9^{Bc}	0.509 ± 0.013^{Aa}
28	35	0	180[5]	———	251.9 ± 4.8^{Bb}	418 ± 11^{Bb}	460 ± 13^{ABbc}[4]	0.387 ± 0.011^{Bb}[4]
29	0	215	0	459	289.4 ± 6.0^{Aa}	458 ± 15^{ABa}	498 ± 12^{Aa}	0.367 ± 0.015^{Bb}
30	0	35	180[5]	———	288.9 ± 7.3^{Aa}	453 ± 10^{ABab}	485 ± 9^{Aab}	0.360 ± 0.012^{Bb}
31	0	0	215	189	294.5 ± 6.2^{Aa}	472 ± 13^{Aa}	497 ± 16^{Aa}	0.364 ± 0.012^{Bb}

[1]Rackis *et al.* (1979).
[2]Level of soy in diet, 30%.
[3]S.E. = standard error, Duncan's Multiple Range Test: Means without a superscript letter in common are significantly different; lower case, $P<0.05$; upper case, $P<0.01$. GBW = grams body weight.
[4]N = 9. Animal, number PAN 17, died at 142 days.
[5]Diet switched to toasted soy diet after 35 days.

group fed toasted soy flour (diet 31). These results, in agreement with previously reported data (Rackis *et al.* 1975A), show that relatively high levels of TI activity can be tolerated by the rat in short- and long-term feeding before the pancreatic hypertrophic properties of the inhibitors will exert a significant biological effect.

DISTRIBUTION OF TRYPSIN INHIBITORS

Natural Occurrence

TI's belong to a broad class of proteins (Protease Inhibitors) that inhibit proteolytic enzymes. Of specific interest is the TI inhibition of trypsin and chymotrypsin, the important animal digestive enzymes for proteins. Protease inhibitors are widely distributed in nature and are present in a large number of plants that are important food sources and are eaten either raw or after cooking. Many popular vegetables eaten in the raw form have relatively high TI activity (Chen and Mitchell 1973), but these TI values cannot be compared directly to soybeans because activity units are not equivalent.

TI Content of Soybeans and Other Food Legumes

Based on an analysis of 57 commercial varieties and advanced breeding lines and plant introductions maintained in the germplasm bank at the U.S. Regional Soybean Laboratory, Urbana, Illinois, the data in Table 12.13 reveal a fourfold variation in TI activity and a twofold variation in chymotrypsin inhibiting activity in soybeans (Kakade *et al.* 1972). Differences in TI activity between field-type and vegetable-type soybeans have been reported by Gupta and Deodhar (1975). However, direct comparisons of relative values obtained by these two groups are not possible because of differences in conditions of analysis and expression of results. According to Valdebouze (1977), it may be possible to make a direct comparison with international inhibitor units (Fritz *et al.* 1974).

Several assays have been used to measure TI activity in soybeans and other foodstuffs and as an index of adequate heat needed to properly process raw soybean meal for maximum nutritive value. A collaborative study by a committee on Soybean Trypsin Inhibitor Analysis assessed some of the problems associated with TI analyses (Rackis *et al.* 1974). A modified procedure, particularly suited for determining insoluble forms of TI in heat-processed and alcohol-treated products, was developed (Kakade *et al.* 1974). As shown in Table 12.14, very good agreement was obtained between two of the original collaborators and analysts in the quality control laboratories of two major soybean processors (Rackis and McGhee 1976).

TABLE 12.13
TRYPSIN INHIBITOR AND CHYMOTRYPSIN INHIBITOR ACTIVITIES OF SEVERAL VARIETIES AND STRAINS OF MATURE SOYBEANS

Parameters	Range of Values	Number of Strains or Varieties	Reference
Trypsin inhibitor activity	66-233[1]	108	Kakade *et al.* (1972)
Trypsin inhibitor activity	33-86[2]	16	Gupta and Deodhar (1975)
Trypsin inhibitor activity	21-66[3]	16	Gupta and Deodhar (1975)
Chymotrypsin inhibitor activity	39-66[4]	26	Kakade *et al.* (1972)

[1]Expressed as trypsin units inhibited/mg protein.
[2]Specific activity units/mg protein, field-type varieties.
[3]Same as [2] above, vegetable-type varieties.
[4]Expressed as chymotrypsin units inhibited/mg protein.

TABLE 12.14
COMPARISON OF TRYPSIN INHIBITOR ACTIVITY OF COMMERCIAL SOY PRODUCTS DETERMINED IN TWO DIFFERENT LABORATORIES

Product	TIU/mg sample[1] USDA[2]	Soy Processor A	Soy Processor B
Isoelectric soy protein isolate (80 PDI)[3]	31.0	35.1	———
Neutralized soy protein isolate (80 PDI)	26.2	23.2	———
Soy protein isolate-III	9.3	9.2	———
Raw defatted soy flour (85 PDI)	72.5	68.1	———
Heated defatted soy flour (25-30 PDI)	4.8	4.3	———
Defatted soy flour (75 PDI)	57.5	———	55.0
Defatted soy flour (11.3 PDI)	6.3	———	6.4

Rackis and McGhee (1976).
[1]TIU = Trypsin inhibitor units as defined by Kakade *et al.* (1969).
[2]Northern Center, Peoria, Ill.
[3]PDI = Protein dispersibility index.

Relative inhibitor activity of various legumes with soybeans as a reference are given in Tables 12.15 and 12.16. Some other food legumes contain low levels of TI activity (0—22 inhibitor trypsin units/mg sample) relative to soybeans (Lopez *et al.* 1978). TI values for extracts represent activity extracted under conditions employed and may not represent total activity of the sample (Rackis *et al.* 1974; Kakade *et al.* 1974).

TABLE 12.15
TRYPSIN INHIBITOR ACTIVITY OF VARIOUS LEGUMES IN RELATION TO SOYBEANS[1]

Legume		Trypsin Inhibitor Activity, %
Scientific Name	Common Names	(Soybeans = 100%)
Vicia faba	Fieldbean, broad bean	2-20
Pisum sativum	*Gardenpea*	5-13
Lupinus species	Lupin beans	0
Vigna sinensis	Cow pea	28

[1]Valdebouze (1977).

TABLE 12.16
TRYPSIN INHIBITOR CONTENT OF FOOD LEGUMES[1]

Legumes	Trypsin Inhibitor Activity $\times 10^{-4}$ units/g
Kidney beans *(Phaseolus vulgaris)*	4.25
Hyacinth beans *(Dolichos lablab)*	4.38
Soybeans *(Glycine max)*	4.15
Lima beans *(Phaseolus lunatus)*	4.04
Pigeon peas *(Cajanus cajan)*	2.77
Cow peas *(Vigna sinensis)*	1.91
Lentils *(Lens esculenta)*	1.78

[1]Liener (1976).

TI Content of Soy Protein Products

TI activity in several commercial heat-processed soy products is given in Table 12.17. Residual TI activity in these products would be well within the tolerance levels established by rat bioassay, when used in diets at high protein levels (see Tables 12.9 and 12.13). A number of soy-based infant formulas also have low levels of TI activity, and these products likewise do not cause pancreatic hypertrophy in rats (Churella *et al.* 1976).

TABLE 12.17
TRYPSIN INHIBITOR (TI) ACTIVITY OF VARIOUS COMMERCIALLY MANUFACTURED SOY PROTEIN PRODUCTS

Product	Trypsin Inhibitor Activity TIU/mg[1]	Mg TI/g Sample[2]	% of Raw Soy Flours	Reference
Raw soy flour[3]	99.0	52.1	100	4
Toasted soy flour[3]	6-15	3.2-7.9	6-15	5
Soybean concentrate	12.0	6.3	12	4
Soybean concentrate	26.5	13.7	27	5
Soybean isolate	8.5	4.4	9	4
Soybean isolate	19.8	10.4	20	4
Soybean isolate	20.9	11.0	21	5
Soy food fiber	12.3	6.5	12	6
Chicken analog	6.9	3.6	7	6
Ham analog	10.2	5.4	10	6
Beef analog	6.5	3.4	7	6
Textured soy flour	9.8	5.2	10	6

[1]TIU = Trypsin inhibitor units as defined by Kakade *et al.* (1969).
[2]Calculated on the basis that 1.90 TIU is equivalent to 1 μg of TI, Kakade *et al.* (1969).
[3]Several lots were analyzed.
[4]Kakade *et al.* (1974).
[5]Rackis, J.J., and J.E. McGhee, unpublished data.
[6]Liener (1975).

SOYBEAN PROTEASE INHIBITORS

Types

Several different forms of protease inhibitors have been isolated from soybeans that inhibit a large number of proteolytic enzymes (Rackis 1972). The two soybean protease inhibitors studied most extensively are: the Kunitz trypsin inhibitor and the Bowman-Birk inhibitor, which can inhibit both trypsin and chymotrypsin. Investigations by Singh *et al.* (1969), Hymowitz and Hadley (1972), Orf and Hymowitz (1977) on the polymorphic nature of SBTI-A_2 (Kunitz inhibitor) revealed the presence of three electrophoretically distinguishable forms designated Ti^1, Ti^2, and Ti^3. Frequency of distribution of the TI variants differs in USDA (Hymowitz 1973) and European soybean germ plasm (Skorupska and Hymowitz, in press).

In spite of differences in level of protease activity and the presence of inhibitor variants, heat treatment was required to obtain maximum nutritive value (Kakade *et al.* 1972).

Five low-molecular-weight (7000—8000) protease inhibitors designated PI I-V were isolated from Tracy soybeans (Hwang *et al.* 1977).

The inhibitors were rich in sulfur-containing amino acids (15—22%). Protease inhibitors I-IV inhibit trypsin and only inhibitor V, which is identical to Bowman-Birk inhibitor, was able to inhibit chymotrypsin. Genetic aspects of protease inhibitors have been reviewed recently (Orf and Hymowitz, in press).

Protease Inhibitors in Germinated Soybeans

The direct use of germinated soybeans in food products holds promise because of their protein content, which is twice that of other food legumes, as well as increased vitamin content, lowered level of flatulence factors, improved functional properties (Rackis 1978), and improved bread-making qualities (Pomeranz *et al.* 1977). Although protease inhibitor content changes during germination, its physiological function in plants is poorly understood (Ryan 1973; Richardson 1977). Most reports on the effect of the germination process on protein quality are contradictory (Rackis 1978). In the raw form, protein quality was very low for immature, mature, and germinated soybeans (see Table 12.4). After heat treatment, nutritive values of soybeans of the same variety at all three stages of maturity are not significantly different (Bates *et al.* 1977).

TI activity was reported to decrease 70% in soybeans germinated 4 days (Bates *et al.* 1977), whereas Collins and Sanders (1976) found little change in TI activity during germination. In these studies, TI values represented activity extracted under the conditions employed and may not represent total activity in the sample (Rackis *et al.* 1974; Kakade *et al.* 1974).

A rocket immunoelectrophoresis system was developed for assay of the Kunitz soybean trypsin, the major TI in soybeans (Freed and Ryan 1978A). Antibodies specific for Kunitz TI were produced by subcutaneous injection of purified inhibitor in rabbits. On a dry weight basis, Kunitz TI activity of Steele soybeans gradually decreased 13% after 9 days germination. Similar decreases in total TI activity as measured by the enzyme assay of Kakade *et al.* (1974) were also observed during germination.

Orf *et al.* (1977) showed that a new form of the Kunitz TI (SBTI-A_2) appeared during germination in six populations of soybeans. Freed and Ryan (1978B) established that the newly formed TI was immunochemically identical to the Kunitz TI. The four low-molecular-weight TI's and the Bowman-Birk inhibitor, designated PI I-V, are rapidly liberated into the leach water of soaked soybeans (Hwang *et al.* 1978). Whether the protease inhibitors PI I-V also undergo modification during germination is not known.

NUTRITIONAL SIGNIFICANCE OF MULTIPLE PROTEASE INHIBITORS IN SOYBEANS

It was previously mentioned (see Tables 12.3 and 12.13) that TI and chymotrypsin inhibitor activities in several soybean varieties differ widely. It was also pointed out that the protease activity is readily destroyed by moist heat treatment (Kakade *et al.* 1972). TI activity in immature, mature, and germinated soybeans is effectively inactivated by toasting, which also results in a great improvement in protein quality as well as the simultaneous elimination of the growth inhibitory and pancreatic effects of raw soybeans (Bates *et al.* 1977; Rackis 1978). A soybean variety with a TI variant that was electrophoretically different from the TI in usual commercial varieties supported better growth when fed to rats in the form of raw meal (Yen *et al.* 1971). Raw meal prepared from a soybean strain PI 157440, which was devoid of Kunitz TI (SBTI-A_2), also inhibited growth and enlarged the pancreas in chicks to a much lesser extent compared to that in chicks fed raw meal prepared from Amsoy 71 soybeans (Bajjalieh *et al.*, in press). Apparently, no heat-stable protease inhibitors are present in soybeans of varying maturity. The protease inhibitor variants are also readily inactivated by moist-heat treatment (Yen *et al.* 1971; Bajjalieh *et al.*, in press). The practical significance of these findings is that protease inhibitors and other antinutritional factors that may arise in raw immature, mature, and germinated soybeans (regardless of variety) can be readily eliminated by ordinary cooking and moist-heat treatment.

RELATIVE DISTRIBUTION OF MULTIPLE PROTEASE INHIBITORS IN SOYBEANS

The Kunitz TI content of various soybean varieties and strains has been reported (Freed and Ryan 1978A). Results are given in Table 12.18. Two Korean varieties, Baik Tae and Kum du, were devoid of the Kunitz TI. The three plant introductions, which were shown to have genetic variants of Kunitz inhibitor SBTI-A_2 (Hymowitz 1973), contain nearly as much TI as in the commercial varieties, Steele and Harosoy soybeans.

According to Freed and Ryan (1978A), Steele soybeans contain 15 mg Kunitz TI per g seed, dry-basis, as measured by rocket immunoelectrophoresis and a total content of 21 mg TI per g seed as measured by the enzymatic procedure of Kakade *et al.* (1974). This latter value was derived on the basis that 1.9 trypsin units inhibited is equivalent to 1 μg of TI (Kakade *et al.* 1969); 39 trypsin units inhibited would be equivalent to 21 mg total TI per g seed (Table 12.18). Therefore, Kunitz soybean TI accounts for 72% of the total TI activity in Steele soybeans.

Soybeans on the average contain 40% protein, thus Steele soybeans

TABLE 12.18
TOTAL TRYPSIN INHIBITOR (TI) AND KUNITZ TI CONTENTS OF SOYBEAN VARIETIES

Variety	Kunitz TI, mg/g Seed		Total TI Content
	Fresh Weight	Dry Weight	mg/g Seed Dry Weight
Steele	12.9	15	21[1]
Harosoy	12.5	———	———
T-245	13.5	———	———
PI 196.172	9.5	———	———
PI 246.367	10.2	———	———
Baik Tae	None detected	———	———
Kum du	None detected	———	———

Freed and Ryan (1978A)
[1]See text for details in calculating this value.

would contain 50 mg TI per g of protein. Content of the low-molecular-weight protease inhibitors, designated types I-V, in Tracy soybeans is 63 mg per g protein (Hwang *et al.* 1978). Assuming similar distribution in both Steele and Tracy soybeans, the various types of protease inhibitors would account for about 11.3% of the protein content of soybeans. Ryan (1973) reported that TI's represent about 6% of protein in soybeans compared to about 10% of the protein in barley and potatoes.

PERSPECTIVE

There are innumerable examples of deleterious and toxic constituents in seed proteins that have been valuable sources of proteins for man. Over the centuries, man has been able to devise procedures to destroy or eliminate these constituents. The most common procedures have involved a combination or extraction, cooking and fermentation. With soybeans, moist-heat treatment is particularly effective in reducing trypsin inhibitor activity below biological threshold levels, as determined by short-term animal bioassay. With present day manufacturing processes, residual trypsin inhibitor activity in edible-grade soy protein products is about 5—20% of the activity originally present in raw soybeans. The question of whether the ingestion of low levels of trypsin inhibitors over a prolonged period would create adverse effects remains unanswered.

Today, emerging technology has resulted in the production of a wide array of soy protein products with improved organoleptic and functional properties, ranging from simple flours to sophisticated spun food analogs. In view of the increased use of soybeans and other vegetable proteins in the human diet, it is important to assess the significance of any

deleterious factors that may still be present in such products due to inadequate processing. Of particular importance are the TI's and protein with low protein digestibility that cause pancreatic hypertrophy in experimental animals. Because of the key role which the pancreas has in the digestion process investigation of other dietary factors that affect pancreatic function becomes important. In terms of practical nutrition one must take into consideration the influence of high-protein and high-fat diets during long-term consumption of TI's. Protein quality and digestibility of proteins in processed foods vary greatly; therefore, effect of animal and vegetable protein per se on pancreatic function needs to be investigated.

Plant protein foodstuffs are usually deficient in the sulfur-containing amino acids, methionine and cystine. TI's of many legumes are quite rich in cystine and may, in fact, account for about 40% of the total cystine content of some legume proteins. Therefore, elimination of TI by breeding may be counterproductive by reducing the total cystine content of the protein that is already limiting in the S-amino acids.

REFERENCES

American Association of Cereal Chemists. 1974. Determination of trypsin inhibitor activity, method 71-10, American Association of Cereal Chemists, St. Paul, Minn., approved Sept. 1974.

American Oil Chemists' Society. 1976. Trypsin inhibitor activity, Method Ba 12-75. In Official and Tentative Methods. American Oil Chemists' Society, Champaign, Ill.

American Oil Chemists' Society. 1979. Proceedings of World Conference on Vegetable Food Proteins, Amsterdam, Netherlands, Oct. 29—Nov. 3, 1978. J. Am. Oil Chem. Soc. *56*, 99—483.

American Soybean Association. 1974. Proc. World Soy Protein Conference, Munich, Germany, 1973. J. Am. Oil Chem. Soc. *51*(1), 6A—204A.

ANDERSON, R.L., RACKIS, J.J. and TALLENT, W.H. 1979. Biologically active substances in soy products. In Soy Protein and Human Nutrition, H.L. Wilcke, O.T. Hopkins and D.H. Waggle (ed.), pp. 209—234. Academic Press, New York.

BAJJALIEH, N.L., ORF, J.H., HYMOWITZ, T. and JENSEN, A.H. Response of young chicks to trypsin inhibitor variant soybeans as sources of dietary protein. Poultry Sci. In press.

BATES, R.P., KNAPP, F.W. and ARAJO, P.E. 1977. Protein quality of green-mature, dry mature and sprouted soybeans. J. Food Sci. *42*, 271—272.

BIELORAI, R., TAMIR, M. and HURWITZ, S. 1977. Amino acid absorption along the intestinal tract of chicks fed heated and raw soybean meal. J. Nutr. *107*, 1775—1778.

BOONVISUT, S. and WHITAKER, J.R. 1976. Effect of heat, amylase, and disulfide cleavage on the in vitro digestibility of soybean proteins. J. Agric. Food Chem. *24*, 1130—1135.

BOOTH, A.N., ROBBINS, D.J., RIBELIN, W.E. and DE EDS, F. 1960. Effect of raw soybean meal and amino acids on pancreatic hypertrophy in rats.

Proc. Soc. Exp. Biol. Med. *104*, 681—683.
BOOTH, A.N., ROBBINS, D.J., RIBELIN, W.E., DE EDS, F., SMITH, A.K. and RACKIS, J.J. 1964. Prolonged pancreatic hypertrophy and reversibility in rats fed raw soybean meal. Proc. Soc. Exp. Biol. Med. *116*, 1067—1069.
BRESSANI, R., ELIAS, L.G. and MOLINA, M.R. 1977. Estudios sobre la digestibilidal de la proteins de varias especies de leguminosos. Arch. Latinoamer. Nutr. *27*, 215—231.
CHANG, H.Y., REECK, G.R. and MITCHELL, H.L. 1978. Alfalfa trypsin inhibitor. J. Agric. Food Chem. *26*, 1463—1464.
CHEN, I. and MITCHELL, H.L. 1973. Trypsin inhibitors in plants. Phytochemistry *12*, 327—330.
CHURELLA, H.R., YAO, B.C. and THOMSON, W.A.B. 1976. Soybean trypsin inhibitor activity of soy infant formulas and its nutritional significance in rats. J. Agric. Food Chem. *24*, 393—397.
COLLINS, J.L. and SANDERS, G.G. 1976. Changes in trypsin inhibitory activity in some soybean varieties during maturation and germination. J. Food Sci. *41*, 168—172.
Commission of the European Communities. 1977. Protein Quality from Leguminous Crops. Luxembourg: Office for Official Publications of the European Communities.
Committee on Food Protection, Food and Nutrition Board. 1973. Toxicants Occurring Naturally in Foods, 2nd Edition. National Research Council, National Academy of Science, Washington, D.C.
CORRING, T. 1974. Feedback regulation of pancreatic secretion in the pig. Ann. Biol. Anim. Biochim. Biophys. *14*, 487—498.
CORRING, T. and BOURDON, D. 1977. Exclusion of pancreatic exocrine secretion from intestine in the pig: Existence of a digestive compensation. J. Nutr. *107*, 1216—1221.
DIJKHOF, J. and POORT, C. 1978. Non-parallel secretion of newly synthesized rat pancreatic proteins after feeding a diet containing raw soybean flour. Biochim. Biophys. Acta *543*, 167—174.
DIJKHOF, J., POORT, S.R. and POORT, C. 1977. Effect of feeding soybean flour containing diets on the protein synthetic pattern of the rat pancreas. J. Nutr. *107*, 1985—1995.
EVANS, R.J. and BAUER, D.H. 1978. Studies of the poor utilization by the rat of methionine and cystine in heated dry bean seed (*Phaseolus vulgaris*). J. Agric. Food Chem. *26*, 779—784.
FIGARELLA, C., NIGRI, G.A. and GUY, O. 1974. In Bayer Symposium on Proteinase Inhibitors, H.Fritz, H. Tschescle, L.J. Greene and E. Truschert (ed.), p. 213. Springer-Verlag, Berlin.
FOLSCH, U.R., WINCKLER, K. and WORMSLEY, K.G. 1974. Effect of a soybean diet on enzyme content and ultrastructure of the rat exocrine pancreas. Digestion *11*, 161—171.
FOLSCH, U.R. and WORMSLEY, K.G. 1974. The pancreatic secretion of enzymes in rats treated with soybean diet. J. Gastroent. *9*, 679—683.
FOLSCH, U.R. and WORMSLEY, K.G. 1976. [^{14}C] Leucine incorporation into proteins of pancreatic juice in rats fed soya-bean flour. Br. J. Nutr. *36*, 281—288.
Food and Agriculture Organization of the United Nations. 1970. Amino acid content of foods, Nutritional Studies No. 24 and Production Yearbook, Vol. 24, FAO, Rome.

FREED, R.C. and RYAN, D.S. 1978A. Changes in Kunitz trypsin inhibitor during germination of soybeans. An immunoelectrophoresis assay system. J. Food Sci. *43*, 1316—1319.
FREED, R.C. and RYAN, D.S. 1978B. Note on modification of the Kunitz soybean trypsin during seed germination. Cereal Chem. *55*, 534—538.
FRITZ, H., TRANTSCHOLD, I. and WERLE, E. 1974. Protease inhibitors. In Methods of Enzymatic Analysis, 2nd Ed., H.U. Bermeyer (ed.). Academic Press, New York.
FUKUSHIMA, D. 1968. Internal structure of 7S and 11S globulin molecules in soybean protein. Cereal Chem. *45*, 203.
GARLICH, J.D. and NESHEIM, M.C. 1966. Relationship of fractions of soybeans and a crystalline soybean trypsin inhibitor to the effects of feeding unheated soybean meal to chicks. J. Nutr. *88*, 100—110.
GERRY, R.W. 1977. Dehydrated soluble potato solids in broiler rations. Poultry Sci. *56*, 1947—1951.
GERTLER, A., BIRK, Y. and BONDI, A. 1967. A comparative study of the nutritional and physiological significance of pure soybean trypsin and of ethanol-extracted soybean meal in chicks and rats. J. Nutr. *91*, 358—370.
GOSS, R.J. 1966. Hypertrophy versus hyperplasia. Science *153*, 1615—1620.
GREEN, G.M. and LYMAN, R.L. 1972. Feedback regulation of pancreatic enzyme secretion as a mechanism for trypsin inhibitor induced hypersecretion in rats. Proc. Soc. Exp. Biol. Med. *140*, 6—12.
GREEN, G.M., OLDS, B.A., MATTHEWS, H. and LYMAN, B.L. 1973. Protein as a regulator of pancreatic enzyme secretion in the rat. Soc. Exp. Biol. Med. *142*, 1162—1167.
GUPTA, A.K. and DEODHAR, A.D. 1975. Variation in trypsin inhibitor activity in soybean (Glycine max.). Indian J. Nutr. Diet. *12*, 81—84.
HWANG, D.L., LIN, K.T.D., YANG, W.K. and FOARD, D.E. 1977. Purification, partial characterization, and immunological relationships of multiple low molecular weight protease inhibitors of soybeans. Biochim. Biophys. Acta *495*, 369—382.
HWANG, D.L., YANG, W.K. and FOARD, D.E. 1978. Rapid release of protease inhibitors from soybeans. Immunochemical quantitation and parallels with lectin. Plant Physiol. *61*, 30—34.
HYMOWITZ, T. 1973. Electrophoretic analysis of SBTI-A_2 in the USDA soybean germplasm collection. Crop Sci. *13*, 420—421.
HYMOWITZ, T. and HADLEY, H.H. 1972. Inheritance of a trypsin inhibitor variant in seed protein of soybeans. Crop Sci. *12*, 197—198.
IHSE, I., LILJA, P. and LUNDQUIST, I. 1977. Feedback regulation of pancreatic enzyme secretion by intestinal trypsin in man. Digestion *15*, 303—308.
JOHNSON, A., HURWITZ, R. and KRETCHMER, N. 1977. Adaptation of rat pancreatic amylase and chymotrypsinogen to changes in diet. J. Nutr. *107*, 87—96.
KAKADE, M.L., HOFFA, D.E. and LIENER, I.E. 1973. Contribution of trypsin inhibitors to the deleterious effects of unheated soybeans fed to rats. J. Nutr. *103*, 1772—1778.
KAKADE, M.L., RACKIS, J.J., MC GHEE, J.E. and PUSKI, G. 1974. Determination of trypsin inhibitor activity of soy products: A collaborative analyses of an improved procedure. Cereal Chem. *51*, 376—382.
KAKADE, M.L., SIMONS, N.R., LIENER, I.E. and LAMBERT, J.W. 1972.

Biochemical and nutritional assessment of different varieties of soybeans. J. Agric. Food Chem. *20*, 87—90.
KAKADE, M.L., SIMONSON, N. and LIENER, I.E. 1969. An evaluation of natural vs synthetic substrates for measuring the antitryptic activity of soybean samples. Cereal Chem. *46*, 518—526.
KAKADE, M.L. *ET AL.* 1976. Failure of soybean trypsin inhibitor to exert deleterious effects in calves. J. Dairy Sci. *59*, 1484—1489.
KONIJN, A.M., BIRK, Y. and GUGGENHEIM, K. 1970. In-vitro synthesis of pancreatic enzymes: Effects of soybean trypsin inhibitors. Am. J. Physiol. *218*, 1108—1112.
KWAAN, H.C., KOK, P. and ASTUP, T. 1968. Impairment of growth and pancreatic hypertrophy in rats fed trypsin inhibitor from raw peanuts. Experientia *24*, 1125—1126.
LEWIS, J.H. and TAYLOR, F.H.L. 1947. Comparative utilization of raw and autoclaved soy bean protein by humans. Proc. Soc. Exp. Biol. Med. *64*, 85—87.
LIENER, I.E. 1976. Legume toxins in relation to protein digestibility. A review. J. Food Sci. *41*, 1076—1081.
LIENER, I.E. 1977. Toxicological considerations in the utilization of new protein foods. In Biochemical Aspects of New Protein Food. J. Adler-Nissen (ed.), p. 129. Pergamon Press, New York.
LIENER, I.E. and KAKADE, M.L. 1969. Protease inhibitors. In Toxic Constituents of Plant Foodstuffs, I.E. Liener (ed.). Academic Press, New York.
LOPEZ, A.S., INFANTE, M.H. and CRUZ, M.E.A. 1978. Trypsin inhibitors and hemagglutinins in certain edible leguminosae. Arch. Invest. Med. (Mex.) *9*, 1—14.
LYMAN, R.L. 1957. Effect of raw soybean meal and trypsin inhibitor diets on the intestinal and pancreatic nitrogen in the rat. J. Nutr. *62*, 285—294.
LYMAN, R.L., WILCOX, S.A. and MONSEN, E.R. 1962. Pancreatic enzyme secretion produced in the rat by trypsin inhibitors. Am. J. Physiol. *202*, 1077—1082.
MADAR, Z., BIRK, Y. and GERTLER, A. 1977. Intestinal absorption of soybean Bowman-Birk trypsin inhibitor. Isr. J. Med. Sci. *13*, 926.
MCNAB, J.M. 1977. Response of broiler chickens to heat treatment of field beans (*Vicia faba* L.). In Protein Quality from Leguminous Crops, pp. 236—242. Commission of the European Communities, Luxembourg: Office for Official Publications of the European Communities.
MELMED, R.N. and BOUCHIER, I.A.D. 1969. Further physiological role for naturally-occurring trypsin inhibitors: Evidence for a trophic stimulant of the pancreatic acinar cell. Gut *10*, 973—979.
MELMED, R.N., EL-AASER, A.A.A. and HOLT, S.J. 1976. Hypertrophy and hyperplasia of the neonated rat exocrine pancreas induced by orally administered soybean trypsin inhibitor. Biochim. Biophys. Acta *421*, 280—288.
MITCHELL, H.L., PARRISH, D.B., CORNEY, M. and WASSOM, C.E. 1976. Effect of corn trypsin inhibitor on growth of rats. J. Agric. Food Chem. *24*, 1254—1255.
NIESS, E., IVY, C.A. and NESHEIM, M.C. 1972. Stimulation of gallbladder emptying and pancreatic secretion in chicks by soybean whey proteins. Proc. Soc. Exp. Biol. Med. *140*, 291—296.
NITSAN, Z. and GERTLER, A. 1972. Enzymatic activities in the intestinal tract of chicks receiving a diet supplemented with trypsin inhibitors from soy-

bean and egg white. Nutr. Metab. *14*, 371—376.
NITSAN, Z. and MADAR, Z. 1978. The level and origin of amylase (E.C. 3.2.1.1) in the digestive tract of chicks receiving trypsin inhibitors in their diet. Br. J. Nutr. *40*, 235—242.
ORF, J.H. and HYMOWITZ, T. 1977. Inheritance of a second SBTI-A_2 variant in seed protein of soybeans. Crop Sci. *17*, 811—813.
ORF, J.H. and HYMOWITZ, T. 1979. Inheritance of the absence of Kunitz trypsin in seed proteins of soybeans. Crop Sci. *19*, 107—109.
ORF, J.H. and HYMOWITZ, T. Genetics of the Kunitz trypsin inhibitor, an antinutritional factor in soybeans. J. Am. Oil Chem. Soc. In press.
ORF, J.H., MIES, D.W. and HYMOWITZ, T. 1977. Qualitative changes of the Kunitz trypsin inhibitor in soybean seeds during germination as detected by electrophoresis. Bot. Gaz. *138*, 255—260.
OSBORNE, T.B. and MENDEL, L.B. 1917. The use of soybeans as food. J. Biol. Chem. *32*, 369—387.
PATTEN, J.R., RICHARDS, E.A. and POPE, H., II. 1971A. The effect of raw soybean on the pancreas of adult dogs. Proc. Soc. Exp. Biol. Med. *137*, 59—63.
PATTEN, J.R., RICHARDS, E.A. and WHEELER, J. 1971B. The effect of dietary soybean trypsin-inhibitor on the histology of the dog pancreas. Life Sci. *10*, 145—150.
PHADKE, K. and SOHONIE, K. 1962. Nutritive value of field bean (*Dolichos Lablab*). V. Nature of possible antigrowth factors. J. Sci. Ind. Res. (India) *21C*, 272—275.
POMERANZ, Y., SHOGREN, M.D. and FINNEY, R.F. 1977. Flour from germinated soybeans in high-protein bread. J. Food Sci. *42*, 824—827; 842.
Protein Advisory Group of the United Nations. 1973. PAG Statement No. 22, PAG Bull., Vol. III, No. 2, United Nations, New York.
RACKIS, J.J. 1965. Physiological properties of soybean trypsin inhibitor and their relationship to pancreatic hypertrophy and growth inhibition of rats. Fed. Proc. *24*, 1488—1493.
RACKIS, J.J. 1972. Biologically-active components. In Soybeans: Chemistry and Technology, Vol. 1, Proteins. A.K. Smith and S.J. Circle (ed.), pp. 158—202. Avi Publishing Co., Westport, Conn.
RACKIS, J.J. 1974. Biological and physiological factors in soybeans. J. Am. Oil Chem. Soc. *51*, 161A—174A.
RACKIS, J.J. 1978. Biochemical changes in soybeans: Maturation, postharvest storage and processing, and germination. In Postharvest Biology and Biotechnology. H.O. Hultin and M. Milner (ed.). Food & Nutrition Press, Westport, Conn.
RACKIS, J.J., HONIG, D.H., SESSA, D.J. and CAVINS, J.R. 1971. Soybean whey proteins: Recovery and amino acid analysis. J. Food Sci. *36*, 10—13.
RACKIS, J.J. and MC GHEE, J.E. 1976. Practical significance of the soybean trypsin inhibitors (Spanish). Proc. 1st Latin-American Soybean Protein Conference, Mexico City, Mexico.
RACKIS, J.J., MC GHEE, J.E. and BOOTH, A.N. 1975A. Biological threshold levels of soybean trypsin inhibitors by rat bioassays. Cereal Chem. *52*, 85—92.
RACKIS, J.J., MC GHEE, J.E., GUMBMANN, M.R. and BOOTH, A.N. 1979. Effects of soy proteins containing trypsin inhibitors on long-term feeding studies in rats. J. Am. Oil Chem. Soc. *56*, 162—168.

RACKIS, J.J., MC GHEE, J.E., HONIG, D.H. and BOOTH, A.N. 1975B. Processing soybeans into foods: Selected aspects of nutritional and flavor. J. Am. Oil Chem. Soc. *52*, 249A—253A.

RACKIS, J.J., MC GHEE, J.E., LIENER, I.E., KAKADE, M.L. and PUSKI, G. 1974. Problems encountered in measuring trypsin inhibitor activity of soy flour. Report of a collaborative analysis. Cereal Sci. Today *19*(11), 513—516.

RACKIS, J.J., SMITH, A.K., NASH, A.M., ROBBINS, D.J. and BOOTH, A.N. 1963. Feeding studies on soybeans. Growth and pancreatic hypertrophy in rats fed soybean meal and fractions. Cereal Chem. *40*, 531—538.

RICHARDSON, M. 1977. The proteinase inhibitors in plants and microorganisms. Phytochemistry *16*, 159—169.

ROMERO, J. and RYAN, D.S. 1978. Susceptibility of the major storage protein of the bean, *Phaseolus vulgaris L.*, to in vitro enzymatic hydrolysis. J. Agric. Food Chem. *26*, 784—788.

RYAN, C.A. 1973. Proteolytic enzymes and their inhibitors in plants. Ann. Rev. Plant Physiol. *24*, 173—196.

SALE, J.K., GOLDBERG, D.M., FAWCETT, A.N. and WORMSLEY, K.G. 1977. Chronic and acute studies indicating absence of exocrine pancreatic feedback inhibition in dogs. Digestion *15*, 540—555.

SAMBETH, W., NESHEIM, M.C. and SERAFIN, J.A. 1967. Separation of soybean whey into fractions with different biological activities for chicks and rats. J. Nutr. *92*, 479—490.

SCHNEEMAN, B.O., CHANG, I., SMITH, L.B. and LYMAN, R.L. 1977. Effect of dietary amino acids, casein, and soybean trypsin inhibitors on pancreatic protein secretion in rats. J. Nutr. *107*, 281—288.

SCHNEEMAN, B.O. and LYMAN, R.L. 1975. Factors involved in the intestinal feedback regulation of pancreatic enzyme secretion in the rat. Proc. Soc. Exp. Biol. Med. *148*, 897—903.

SEIDL, D., JAFFE, M. and JAFFE, W.G. 1969. Digestibility and proteinase inhibitory action of a kidney bean globulin. J. Agric. Food Chem. *17*, 1318—1321.

SINGH, L., WILSON, C.M. and HADLEY, H.H. 1969. Genetic differences in soybean trypsin inhibitors separated by disc electrophoresis. Crop Sci. *9*, 489—491.

SKORUPSKA, H. and HYMOWITZ, T. On the frequency distribution of celleles of two seed proteins in European soybean (*Glycine max.* L. Merrill) germplasm: Implications on the origin of European soybean germ plasm. Genet. Pol. In press.

SMITH, A.K. and CIRCLE, S.J. (ed.). 1972. Soybeans. Chemistry and Technology, Vol. 1, Proteins. Avi Publishing Co., Westport, Conn.

SMITH, A.K., RACKIS, J.J., MC KINNEY, L.L., ROBBINS, D.J. and BOOTH, A.N. 1964. Growth and pancreatic hypertrophy in rats fed laboratory and commercial soybean meal and hulls. Feedstuffs *36*, 46—49.

SNOOK, J.T. 1968. Factors in whole egg protein influencing dietary induction of increases in enzyme and RNA levels in rat pancreas. J. Nutr. *97*, 286—294.

SWARTZ, M.J., MITCHELL, H.L., COX, D.J. and REECK, G.R. 1977. Isolation and characterization of trypsin inhibitor from opaque-2-corn seeds. J. Biol. Chem. *252*, 8105—8107.

TASHIRO, M. and MAKI, Z. 1978. Partial purification and some properties of a trypsin inhibitor from rice bran. Agric. Biol. Chem. *42*, 1119—1124.

THOMPSON, R.M. and LIENER, I.E. 1978. Resistance of navy beans

(*Phaseolus vulgaris*) storage protein to digestion. Fed. Proc. *37*, Abstr. No. 278.

VALDEBOUZE, P. 1977. Trypsin inhibitor and hemagglutinating activities in seeds of some legume species. In Protein Quality from Leguminous Crops, pp. 87—98. Commission of the European Communities, Luxembourg; Office for Official Publications of the European Communities.

WILCKE, H.L., HOPKINS, D.T. and WAGGLE, D.H. (ed.). 1979. Soy Protein and Human Nutrition. Academic Press, New York.

WILSON, P.A., MELMED, R.N., HAMPE, M.V. and HOLT, S.J. 1978. Immunocytochemical study of the interactions of soybean trypsin inhibitor with rat intestinal mucosa. Gut *19*, 260—265.

YEN, J.T., HYMOWITZ, T. and JENSEN, A.H. 1971. Utilization by rats of protein from a trypsin inhibitor variant soybean. J. Anim. Sci. *33*, 1012—1017.

YEN, J.T., JENSEN, A.H. and SIMON, J. 1977. Effect of dietary raw soybean and soybean trypsin inhibitor on trypsin and chymotrypsin activities in the pancreas and in small intestinal juice of growing swine. J. Nutr. *107*, 156—165.